BLOOD CHANGES IN ACUTELY SPLENECTOMIZED RATS DURING PROLONGED HYPEROXIC EXPOSURE

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It is generally accepted that changes in the circulating red cell mass can be effected by O_2 acting on the rate of erythropoiesis. This applies both to O_2 partial pressures greater than ambient which suppress RBC production and to hypoxia which stimulates this process. The possibility that O_2 can alter the rate of hemolysis has also been considered. Thus, O_2 at high pressure has been shown to increase hemolysis *in vivo* in tocopherol deficient mice (1). But, as Kaplan pointed out (2), the question as to whether moderate increases in O_2 can increase hemolysis in otherwise normal animals or humans remains largely unanswered.

The spleen is a major site of RBC destruction as well as storage. Considerable evidence suggests that the spleen can selectively sequester the older more fragile RBC's. Thus, it is probable that a sampling procedure that causes splenic contraction could rapidly change both the qualitative and the quantitative nature of the circulating red cell mass. In this latter regard, it is well demonstrated that splenic contraction induced by epinephrine can increase the total blood volume by 6-15% with a disproportionately greater effect on the hematocrit due to the concentration of the discharge.

It was felt that any effect of O_2 on circulating RBC's would be more evident in splenectomized rats. The experiments reported herein, which are preliminary, show that with such rats, consistent changes in blood, indicative of an increased hemolytic process, can be effected by exposure to O_2 at partial pressures below 1 atm.

METHODS

Male S-D rats weighing 250 gm were splenectomized 4 days prior to exposure. In an initial series of experiments, groups of 6 rats were exposed to 600 mm Hg O_2 in individual chambers (3) for 0, 2, 4, 8, 14, and 28 days. These were compared to an equal number of pair-fed, splenectomized air-control rats kept in metabolic cages. Hematocrit, hemoglobin, methemoglobin, serum bilirubin, and osmotic fragility curves were determined on both groups at the stated intervals. In another series, rats similarly treated were exposed to 600, 450, 258, or 190 mm Hg O_2 for 14 or 28 days. These were compared to both splenectomized or sham-operated pair-fed controls. Hematocrit, hemoglobin, and osmotic fragilities were determined. RBC reduced glutathione content and dialuric acid hemolysis, a measure of antioxidant reserves, were also measured.

RESULTS

The results of the 600 mm Hg O_2 exposure study are illustrated in Figures 1 and 2. The hematocrit in Figure 1 is significantly depressed after 2 days (38 vs. 42%). This trend is still evident after 28 days. Congruent changes in the total Hb of the exposed rats suggest a relative RBC loss. Methb and bilirubin showed no significant deviations from those of the control rats.

Figure 2 illustrates the significant fragility changes during the 28-day exposure. The

SPLENECTOMIZED ♂ S.D. RATS EXPOSED TO 600 mm Hg O_2

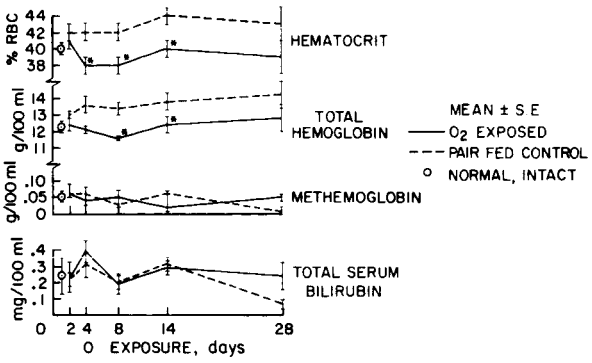

Figure 1

ERYTHROCYTE FRAGILITY OF SPLENECTOMIZED ♂ S.D. RATS EXPOSED TO 600 mm Hg O_2

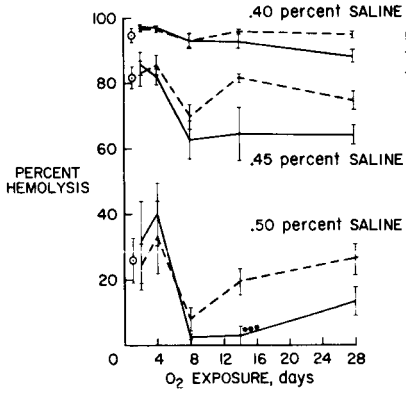

Figure 2

O_2-exposed rats show sizable *decreases* in fragility by the eighth day. In another series, significant decreases were noted after only 3 days of exposure to 600 mm Hg. As in the case of the hematocrits and Hb content, the changes tend to persist for at least 28 days.

Osmotic fragility was determined in saline concentrations from 0.2% which caused 100% hemolysis to 0.55% where lysis was negligible. However, the greatest decreases in fragility occurred in the higher saline concentrations.

The established relationships between osmotic fragility, volume, and age of RBC's (4) suggest that the above changes in fragility are due to a relative lack of older, more fragile cells found in a normal population. In this regard, it is interesting that a significant decrease in reticulocytes was seen only after 14 days and was no longer evident after 28 days.

The hematocrit (Fig. 3) was significantly suppressed by exposure to 258 mm Hg after 2 weeks and to 450 mm Hg for 4 weeks. At neither time interval was the hematocrit depressed by exposure to 190 mm Hg O_2 which gives an alveolar pO_2 just below normal. Hb content (Fig. 4) showed a similar trend, and was significantly depressed at

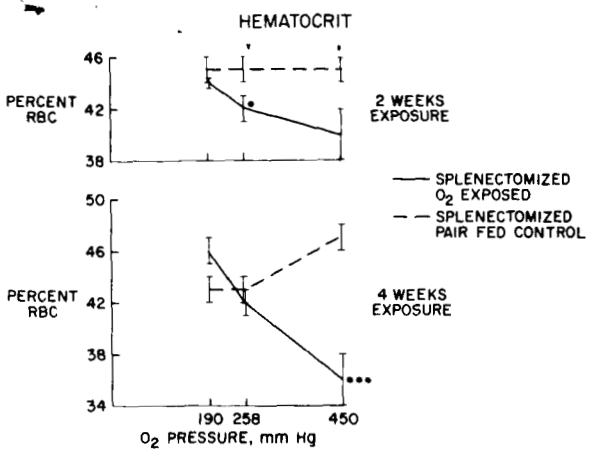

Figure 3

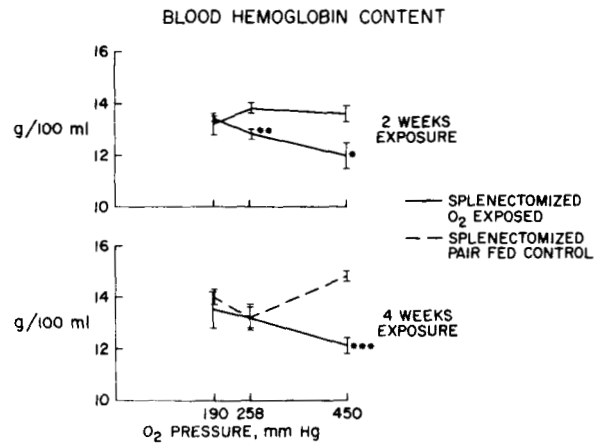

Figure 4

both 258 and 450 mm Hg after 2 weeks and at 450 mm Hg after 4 weeks. Again, no differences were observed between the 190 mm Hg group and its controls.

In this series, the greatest fragility changes occurred in 0.40% saline. At both 258 and 450 mm Hg O_2 osmotic fragility was significantly reduced after 2 weeks of exposure (Fig. 5). On the other hand, fragility at all three O_2 pressures is essentially similar to that of the air controls after 4 weeks of exposure. Thus, the fragility changes induced initially by exposure to O_2 at 258 or 450 mm Hg appear self-limiting; in fact, pre-exposure fragilities appear to be reestablished after 4 weeks despite continued exposure.

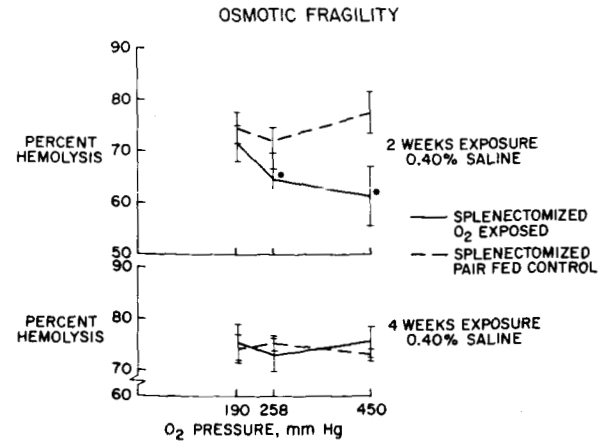

Figure 5

DISCUSSION

The very nature of splenic function would suggest that sequestered cells are of an older population distribution than the circulating blood; therefore, splenic contraction would tend to increase the proportion of older more fragile cells in the circulation and would increase the hematocrit. The occurrence of this event just prior to or during a sampling procedure could lead to an erroneous conclusion concerning the effects of some previous condition on the blood.

It is recognized that a decrease in hematocrit or Hb alone is not sufficient evidence to verify a decrease in red cell mass, and these techniques were employed merely as indicators.

The consistent decreases in fragility indicate strongly that exposure to a pO_2 as low as 258 mm Hg somehow stimulates the removal of the more fragile cells, for if O_2 were merely to suppress hemopoiesis, one would not expect to see a decrease in the older more fragile cells. On the other hand, if peroxidative hemolysis were occurring in the face of continued O_2 exposure, RBC's would be expected to show a greater fragility. In this regard, no evidence of increased dialuric acid hemolysis could be demonstrated even after 10 days of exposure at 600 mm Hg O_2.

SUMMARY

Exposing splenectomized rats to O_2 at pressures from 600 to 258 mm Hg consistently changes hematocrit, total Hb, and osmotic fragilities of RBC's, indicating that the circulating red cell mass has been decreased by an active removal of the older or more fragile cells. These changes have been observed within 3 days but appear self-limiting.

REFERENCES

1. Mengel, C. E. and Kann, H. E., Jr.: *J. Clin. Invest.* 45:1150, 1966.
2. Kaplan, H. P.: *Aerospace Med.* 38:676, 1967.
3. Brooksby, G. A., Dennis, R. L., and Staley, R. W.: *Proc. Third International Conference on Hyperbaric Medicine*, Natl. Acad. Sci., Washington, D.C., 1966, p. 208.
4. Danon, D. and Perk, K.: *J. Cellular Comp. Physiol.* 59:117, 1962.

Note:

* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$